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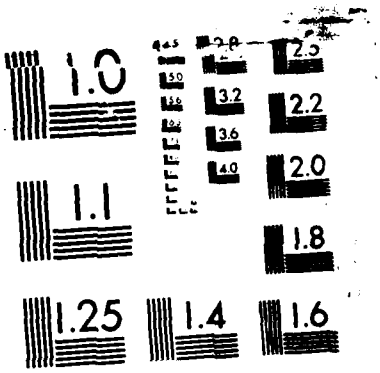

Pardaxin's action in shark

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OBJECTIVES

Structure and mode of action of the shark repellent pardaxin

ABSTRACT

Chemical Characterization of Pardaxin We have purified the main proteinaceous factor (Pardaxin) from the shark-repellent secretion of the Moses sole (Pardachirus marmoratus) fish. Recently Tashibana et al. ~~(Science 226, 703-705, 1984)~~ show that the Moses sole secretion in addition to bioactive proteins also possesses a group of steroid aminoglycosides (sterols).

Those sterols displayed surfactant, hemolytic, toxic and shark repellent activities. They share similar activities described for Pardaxin (PX).

Therefore, we were concerned whether those sterols are associated with PX and are involved in some of its biological activities. We used mass spectroscopy to identify the sterols. Several sterols were identified in the Moses sole secretion, those composed of 2-5% of the protein content. However, mass fragmentation spectra (of 30 mg.) of the PX do not show the presence of such sterols.

Fluorometric analysis of pore formation in lipsomes. The recovery of the valinomycin-induced decrease of fluorescence produced by PX provides a sensitive assay (10^{-10} M - 10^{-9} M) which was also used to assess the pore (channel) activity of PX. The pore activity was expressed within one minute. This effect was observed in the presence and the absence of Na^+, SO_4^- and sucrose indicating the lack of specificity in transporting these solutes. In contrast, using the pore activity assay based on the differential transport of Cl^- and Rb^+, the accumulation of Rb^+ in liposomes treated with PX could be achieved only if more K^+ than Cl^- ions were transported from liposomes to the medium. Here, PX activity is characterized by a long time-course of about 10-15 min. We do not

know the biological significance of a fast nonspecific and slow-action specific pores. Although the structural details of the PX pore require further elucidation, it is tempting to correlate the agregation of PX observed in solution with the number of 4-6 molecules which form an active pore. The observation that small liposomes aggregate after PX treatment also suggest some PX mediated vesicular fusion.

Mechanism of membrane activity. It appears that PX exerts its cytotoxic effects by at least two mechanisms depending on concentration and time of exposure. At low concentrations (lower than 10^{-7} M) it permeabilizes cell membrane to cations, triggering cell death by a pathway shared in common with a variety of other cytolysins. At higher concentration (10^{-7} M - 10^{-4} M) most probably PX acts like a surface protein or detergent, causing cell destruction by rapid dissolution of cell membranes into mixed micelles. Although, it is quite conceivable that the same mechanism of pore formation underlies PX toxicity or repellancy to fish, we cannot exclude the existance of specific receptors on the surface (external) side of the gills, which has been found to be the target organ of Moses sole secretion (Primor, Experientia 41, 693-696, 1985.). The molecular mechanisms of pardaxin interaction with artificial and biological membranes is under investigation. Nevertheless, the present study shows that the toxic principal component of the flat fish secretion is a protein and was obtained free of steroid aminoglycosides. In addition, PX displays unique hydrophobic and amphipathic properties and has the capability of binding to liposomes with the formation of ion permeable pores.

Published work on the subject (1986)

Primor, N. Action on ileal smooth muscle of synthetic detergents and pardaxin. Gen. Pharmacol. In Press.

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